Nerve fibers and menstrual cycle in peritoneal endometriosis

There was no difference in the density of nerve fibers across the menstrual cycle in peritoneal endometriotic lesions. These findings may explain why patients with peritoneal endometriosis often have painful symptoms throughout the menstrual cycle. (Fertil Steril® 2011; — — —. ©2011 by American Society for Reproductive Medicine.)

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Endometriosis can often cause dysmenorrhea, dyspareunia, noncyclical pelvic pain, and subfertility (1). The fundamental pathologic mechanism underlying endometriosis-associated pain still remains unclear. Berkley et al. (2, 3) have shown that ectopic endometriotic implants induce their own sensory and sympathetic nerve supply in rats and rodents. We have also demonstrated nerve fibers in peritoneal endometriotic lesions from patients with endometriosis (4). Yao et al. (5) have shown that patients with endometriosis who have painful symptoms had more nerve fibers in peritoneal endometriotic lesions than patients with endometriosis who had no pain. Mechsner et al. (6) also have shown the correlation between pain score and the density of nerve fibers in peritoneal endometriotic lesions from patients with endometriosis. However, it still unknown whether the density of nerve fibers in peritoneal endometriotic lesions can vary across the menstrual cycle; therefore, we decided to explore the density of nerve fibers in peritoneal endometriosis in the menstrual, proliferative, and secretory phases.

This study was approved by the Human Ethics Committees of the Sydney South West Area Health Service and the University of Sydney, and all women gave their informed consent for participation. In this study, we studied the numbers of nerve fibers in the menstrual (n = 5), proliferative (n = 19), and secretory (n = 20) phases in 44 patients with laparoscopic evidence of endometriosis, and those subjects had a range of pain symptoms, such as dysmenorrhea and dyspareunia (mean age 32.3 years, range 22–48 years). The severity of pain was not assessed systematically or prospectively in this study, but detailed clinical information was recorded in a standard format. Endometriosis in all patients was staged according to the revised American Fertility Society score, which ranged from 1 to 4. We used protein gene product 9.5 (PGP9.5) and neurofilament (NF) to investigate the presence of myelinated and unmyelinated nerve fibers in peritoneal endometriotic lesions. Specimens were immediately fixed in 10% neutral buffered formalin for approximately 18–24 hours, processed, and embedded in paraffin according to a standard protocol. Each section was cut at 4 μm and routinely stained with hematoxylin and eosin. Serial sections, cut at 4 μm, were heat-retrieved with Target retrieval solution (pH 9.0 for PGP9.5 and pH 6.0 for NF) for 20 minutes at 100°C. All slides were immunostained for polyclonal rabbit anti-PGP9.5 (dilution 1:1,400), a highly specific pan-neuronal marker that recognizes all types of nerve fibers, and monoclonal mouse anti-human NF (dilution 1:800), a highly specific marker for myelinated nerve fibers, for 30 minutes; incubated with the REAL Detection System (Dako, Australia), alkaline phosphatase/RED, biotinylated secondary antibodies, and streptavidin alkaline phosphatase for 15 minutes; and stained with the REAL Detection System, chromogen (RED) for 10 minutes. We used normal skin as a positive control because it reliably contains myelinated and unmyelinated nerve fibers expressing PGP9.5 and NF; the mean (±SD) density of nerve fibers stained with PGP9.5 was 12.5 ± 2.6/mm². Rabbit immunoglobulin fraction was used as a negative control, the concentration being matched with the concentration of the antibodies.

All immunostaining was carried out on a Dako Autostainer, model S3400 (Dako, Carpinteria, CA). Images of the sections were captured using an Olympus microscope BX51 and digital camera DP70 (Olympus, Tokyo, Japan), and an assessment of nerve fiber density was performed by Image Pro Plus Discovery (Media Cybernetics, Bethesda, MD). The counting procedure...
The density of nerve fibers stained with PGP9.5 in the menstrual phase was $15.7 \pm 12.6/mm^2$ (range, 5.5–36.8/mm²), in the proliferative phase was $14.9 \pm 11.2/mm^2$ (range, 5.5–46/mm²), and in the secretory phase was $15.9 \pm 10.2/mm^2$ (range, 3.7–44.2/mm²). There were no differences in the density of nerve fibers at different stages of the menstrual cycle ($P > 0.05$) (Fig. 1).

The density of nerve fibers stained with NF in the menstrual phase was $5.7 \pm 5.6/mm^2$ (range, 1.8–16.6/mm²), in the proliferative phase was $5.2 \pm 4.7/mm^2$ (range, 1.8–16.6/mm²), and in the secretory phase was $5.4 \pm 5.2/mm^2$ (range, 1.8–14.7/mm²). There were no differences in the density of nerve fibers across the menstrual cycle ($P > 0.05$).

We have demonstrated multiple small unmyelinated nerve fibers in peritoneal endometriotic lesions, and there were many more nerve fibers in the peritoneal endometriotic lesions (mean 16.3 ± 10.0/mm²) than in normal peritoneum from women without endometriosis (mean 2.5 ± 1.3/mm²; $P < 0.001$) (4). We have also found that the nerve fiber density in peritoneal endometriotic lesions from hormonally treated women with endometriosis (10.6 ± 2.2/mm²) was significantly lower than in peritoneal endometriotic lesions from hormonally untreated women with endometriosis (16.3 ± 10.0/mm²) (7). Yao et al. (5) have found PGP9.5-immunoreactive nerve fibers in 62% of peritoneal endometriotic lesions from patients with endometriosis with pain, and the density was $3.8 \pm 1.7/mm^2$, which was significantly higher than that in endometriosis without pain ($1.7 \pm 0.5/mm^2; P < 0.05$). Mechsner et al. (6) have shown that peritoneal endometriosis-associated nerve fibers were found significantly more frequently in a group with a pain score of at least 3 or more than in a group with a pain score of 2 or less. The same group has also reported that nerve fibers stained with growth-associated protein 43, a marker of neural outgrowth, were expressed only in nerve fibers in peritoneal endometriotic lesions and not in normal peritoneum (8). These studies support the hypothesis that nerve fibers play an important role in the etiology of endometriosis-associated pain.

To the best our knowledge, this is the first study evaluating the density of nerve fibers at different stages of the menstrual cycle in peritoneal endometriotic lesions. The data showed that there was no difference in the density of nerve fibers among the menstrual, proliferative, and secretory phases in peritoneal endometriotic lesions. These findings may help to explain why patients with endometriosis often have painful symptoms throughout the menstrual cycle.
REFERENCES